Biodegradation (2020) 31:47–56
https://doi.org/10.1007/s10532-020-09893-z

ORIGINAL PAPER

Study and valorisation of wastewaters generated in the production of bacterial nanocellulose

Francisco A. G. Soares da Silva · João V. Oliveira · Catarina Felgueiras · Fernando Dourado · Miguel Gama · M. Madalena Alves

Received: 26 November 2019 / Accepted: 5 March 2020 / Published online: 19 March 2020

Abstract Two culture media were tested for the production of bacterial nanocellulose (BNC) under static culture fermentation, one containing molasses (Mol-HS), the other molasses and corn steep liquor (Mol-CSL), as a source of carbon and nitrogen, respectively. These are low-cost nutrients widely available, which provide very good BNC productivities. However, the use of these substrates generates wastewaters with high organic loads. Anaerobic digestion is one of the most promising treatments for industrial wastewaters with high organic loads since, beyond removal of the organic matter, it generates energy, in form of biogas. The wastewaters from BNC fermentation were thus evaluated for their biochemical methane potential through anaerobic digestion. For this, two wastewaters streams were collected: (i) the culture medium obtained after fermentation (WaF) and (ii) the WaF combined with BNC washing wastewaters (WaW). These two effluents—WaF and WaW—were characterized regarding their chemical oxygen demand, total nitrogen, total and volatile solids, to assess their suitability for anaerobic digestion. The biochemical methane potential of WaF and WaW from Mol-CSL wastewaters was (387 ± 14 L kg^{-1} VS) and (354 ± 4 L kg^{-1} VS), corresponding to a methanization percentage of (86.9 ± 3.1) % and (79.5 ± 0.9) %, respectively. After treatment, the chemical oxygen demand of WaF and WaW was reduced by (89.2 ± 0.4) and (88.7 ± 1.5), respectively. An exploratory test using an Upflow Anaerobic Sludge Blanket reactor for WaW treatment was also performed. The reactor was operated with a organic loading rate of [(6.5 ± 0.1) g L^{-1} d^{-1}] and hydraulic retention time of 3.33 days, allowing a chemical oxygen demand removal of 58% of WaW. Results here obtained demonstrate, for the first time, the high potential of AD for the valorisation of the BNC fermentation wastewaters.

Keywords Bacterial cellulose · Wastewater · Anaerobic digestion · Biogas

F. A. G. S. da Silva · J. V. Oliveira · C. Felgueiras · F. Dourado (✉) · M. Gama · M. M. Alves
Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
e-mail: fdourado@deb.uminho.pt

Introduction

Bacterial nanocellulose (BNC) is a 3D nanofibrillar cellulose network, produced by acetic acid bacteria. The membranes produced in static culture present high porosity, high water retention capacity, high mechanical strength in the wet state, and biocompatibility (Jozala et al. 2016). However, the large-scale BNC production remains a challenge due to the low yields, ineffective fermentation systems, high capital investment and high operating costs (Gama et al. 2016). As with many fermentation processes, the cost and availability of the substrates also play a determining

role in the economic feasibility of the process (Jozala et al. 2016). The sustainable BNC fermentation from low-cost organic by-products has long been pursued. Examples include the use of waste glycerol from biodiesel (Vazquez et al. 2013), molasses (Bae and Shoda 2004; Premjet et al. 2007; Rodrigues et al. 2018), grape bagasse (Vazquez et al. 2013), wheat straw (Chen et al. 2013), corn stalk (Cheng et al. 2017) and corn steep liquor (CSL) (Costa et al. 2017). Other alternative substrates for the BNC production include distillery effluents from several industries as pulp and paper or beverage, and wastes from textile and agricultural production (Kongruang 2007; Lin et al. 2014; Tsouko et al. 2015; Campano et al. 2016; Huang et al. 2016; Zhao et al. 2018).

Despite their low cost and high BNC yields, these low value-added nutrient sources contain high organic loads, some also having antimicrobial components such as phenols, which are major drawbacks for the BNC fermentation processes. Indeed, these alternative substrates may place an economic burden either downhill, due to the need for more demanding wastewaters treatments and/or, uphill, due to the need of substrates pre-treatment before fermentation. Therefore, while the BNC productivity is important, the overall economic and environmental impact of using alternative substrates for the BNC fermentation should be considered for the economic but sustainable production of BNC. To the authors knowledge, no work has been done on the characterization of the wastewaters from the BNC production process. Further, the valorisation of the BNC fermentation and purification wastewaters through anaerobic digestion is here addressed, specifically concerning their potential for biogas production, through anaerobic digestion.

Anaerobic digestion (AD) is an effective microbiological wastewater pre-treatment process, offering meaningful advantages including low sludge production, low energy requirement, odour reduction, control of pathogens or renewable energy recovery while treating wastewaters with high organic loads (Mata-Alvarez et al. 2000; Botheju and Bakke 2011; Rajagopal et al. 2013). Through AD, organic matter is upcycled into biogas (about 70% methane and 30% of carbon dioxide), which can be used for heat and/or electricity generation (Wilkie 2005; Appels et al. 2008). As a renewable energy source recovered from organic waste, biogas has been receiving increasing attention over the past few years. Numerous studies were reported on all aspects of biogas production, from processing to utilization (Dupla et al. 2004; Mata-Alvarez et al. 2000; Massé et al. 2010; Zhang et al. 2007).

In this work, a synthetic medium (as a control) and alternative low-cost and high organic load substrates were used for the BNC fermentation under static culture. The resulting wastewaters from each assay were characterized regarding chemical oxygen demand (COD), total nitrogen (TN), total and volatile solids (TS and VS), to assess their suitability for AD. Afterwards, AD was studied in batch and in continuous assays, to evaluate the upcycling of the organic wastes into biogas.

Material and methods

Bacterial nanocellulose fermentation

Bacterial strain and culture medium

The strain *Komagataeibacter xylinus ATCC 700178* was used for the BNC production. The strain was maintained in Hestrin-Schramm culture medium (HS) with agar (20 g L^{-1}) (HIMEDIA) (Schramm and Hestrin 1954). Different liquid culture formulations were tested for BNC (and effluent) production: a modified synthetic HS medium (Schramm and Hestrin 1954) and two alternative media containing molasses, supplied by RAR (Refinarias de Açucar Reunidas. S.A.; Portugal) as carbon source and Corn Steep Liquor (CSL), supplied by COPAM (Companhia Portuguesa de Amidos. S.A.; Portugal), as nitrogen source. In previous work, the optimization of BNC production by *K. xylinus ATCC 700178* (BPR2001) using these nutrients was performed (Rodrigues et al. 2018). In this work, the impact of molasses and CSL on the wastewater organic loads was assessed. The effluents generated in the BNC production using the following culture media were collected:

- HS (control culture medium)—Glucose 40.0 g L^{-1} (Fischer chemical); peptone 5.0 g L^{-1} (Liofilchem); yeast extract 5.0 g L^{-1} (Liofilchem);
- Mol-HS, where the glucose content in HS was replaced with an equivalent amount of molasses (40.0 g L^{-1} of total sugars)

- Mol-CSL, where the glucose content in HS was replaced with an equivalent amount of molasses (40.0 g L^{-1} of total sugars); the protein content was replaced with CSL (7.0 g L^{-1}); ammonium sulphate (0.5% (w/v)) was also added.

All formulations were complemented with ethanol 1.5% (v/v), disodium phosphate di-hydrated 3.39 g L^{-1} (Labkem) and citric acid 1.26 g L^{-1} (Panreac) (Rodrigues et al. 2018). The composition of molasses (determined as described on "Analytical methods" section) was: sucrose 625 g L^{-1}; glucose 19 g L^{-1}; and fructose 11 g L^{-1}. Total protein in CSL (determined as described on "Analytical methods" section) was of (177.1 ± 7.0) g L^{-1}.

Inoculum preparation and static culture fermentation

Komagataeibacter xylinus cells were grown under static culture, in 1 L conical flasks with 100 mL of HS medium, containing the following components (in g L^{-1}): glucose 20.0, peptone 5.0, yeast extract 5.0, disodium phosphate di-hydrated 3.39 and citric acid 1.26. Before inoculation, HS medium was autoclaved at 121 °C for 20 min. After 48 h of incubation of the pre-inoculum, the formed cellulose pellicle was shaken to release the bacteria entrapped within the cellulose matrix into the residual medium, which was used for further inoculation at 10% (v/v) of the final fermentation volume, in the different culture media. These culture media were incubated at 30 °C for 7 days (or 30 days for UASB assay) (in vessels filled up to 1 cm depth) in a static incubator (Pol-EKO Aparatura CLN 180).

Bacterial nanocellulose yield and collection of the wastewaters

After 7 days of fermentation, the produced BNC was milled and washed in order to remove impurities such as culture medium residues and trapped cells. One batch (100 g) of the milled BNC was filtered and the filtrate collected (Wastewater after Fermentation—WaF). (ii) In parallel, another batch of milled BNC was filtered, and the fibres were submitted to a sequential filtration-washing as follows: (i) resuspension in a solution of NaOH 0.1 M (added up to a total mass of 100 g) and filtration; this process was repeated with (ii) NaOH 0.1 M and (iii) distilled water. The filtrates obtained were combined and neutralized with acetic acid (4% v/v) (Wastewater after Washing—WaW). The WaF and WaW filtrates were frozen at − 20 °C until characterization of the COD, TN, total sulphates (TSO_4), TS, VS, volatile fatty acids (VFA) and pH. The washed BNC cake was dried in an oven at 60 °C until constant mass and weighed in order to calculate the volumetric yield of BNC, which was determined by:

$$\frac{\text{BNC}}{\text{g L}^{-1}} = \frac{\text{Dried BC/g}}{\text{Culture medium volume/L}} \quad (1)$$

Anaerobic treatment

Biochemical methane potential (BMP) assays

The BMP assays were performed as described elsewhere (Angelidaki et al. 2009; Holliger et al. 2016). Briefly, the assays were carried in 600 mL serum bottles, using a working volume of 150 mL consisting of inoculum (60 mL, further described below), substrate (1 g of COD in each bottle) and buffer medium according to Holliger et al. (2016). Bottles were closed with rubber stoppers and aluminium caps and the headspace was flushed with N_2/CO_2 (80/20 v/v). A blank assay (without substrate) was used to estimate the methane produced by the inoculum itself. A positive control assay, with microcrystalline cellulose as substrate, was used to access the quality of the inoculum. All assays were performed in triplicates, at 37 °C, with manual stirring, once a day.

The anaerobic inoculum used in the BMP assays consisted of a mixture (50/50 m/m) of anaerobic granular sludge, from a brewery wastewater treatment plant, and fresh manure, from a dairy farm. This mixture was incubated at 37 °C for 15 days, to deplete the residual substrate (degassing process). VS content was of (61 ± 1) g $kg^{-1}$of inoculum.

During the assays, the methane accumulated in the bottles' headspace was measured periodically by gas chromatography (GC), sampling 500 µL with a gas tight syringe. Methane volume was corrected for STP conditions (0 °C and 101.3 kPa) (Costa et al. 2012a). BMP was calculated according to (Costa et al. 2012b) and expressed as the volume of methane at STP conditions per amount of VS of inoculum (L kg^{-1}). The percentage of methanization (PM) was

determined with Eq. 2, where COD_{CH4} represents the COD (g) converted to methane (subtracting the methane produced in the blank assay); $COD_{initial}$ is the COD (g) of the substrate in each bottle.

$$\frac{PM}{\%} = \frac{COD_{CH4}}{COD_{initial}} \times 100 \quad (2)$$

Continuous reactor setup

This trial was performed only with WaW from Mol-CSL medium, as it corresponds to the whole wastewater generated from the BNC production with highest yield. The continuous assay was carried out in a 200 mL acrylic upflow anaerobic sludge blanket (UASB) reactor, with a working volume of 180 mL (Fig. 1). It was operated at 37 °C and inoculated with 10 g L^{-1} of VS. WaW was previously centrifuged at 11,200×*g* for 10 min (Eppendorf 5430 R, rotor F-35-6-30). Sodium bicarbonate was added at 5.0 g L^{-1} to provide suitable alkalinity. The anaerobic digestion of WaW was performed using the following operational parameters (Table 1)

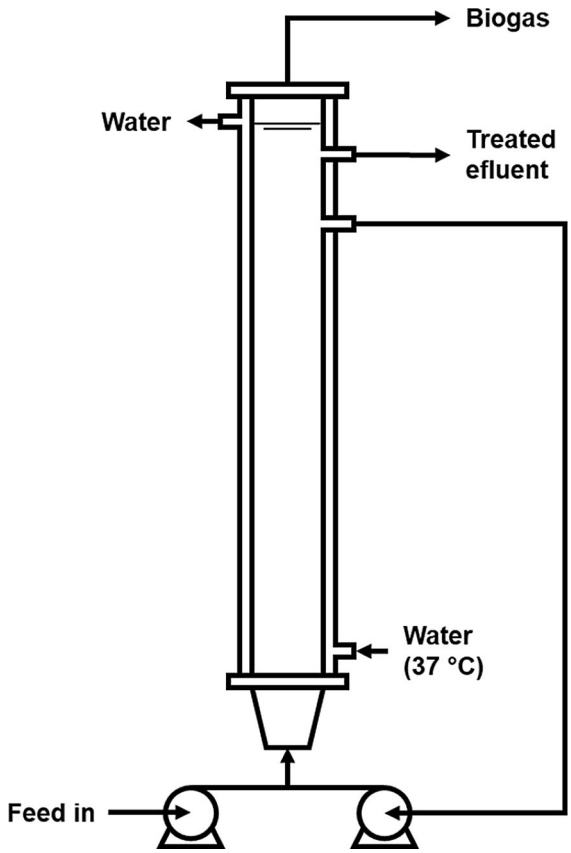

Fig. 1 Scheme of the Upflow Anaerobic Sludge Blanket reactor

Biogas production was measured with a Ritter MilliGas counter (Dr. Ing. Ritter Apparatebau GmbH, Bochum, Germany). Methane content of the biogas was analyzed by GC. Total and soluble COD, pH, VS, sulphide, ammonia and VFA were monitored during reactor operation.

Analytical methods

COD, sulphates and sulphide were determined using standard kits (*Hach Lange*, Düsseldorf, Germany). For soluble COD, the samples were previously centrifuged for 10 min at 25,200×*g* (Eppendorf 5430 R, rotor F-35-6-30). VS (the weight loss after a sample is ignited and heated to dryness) and TS (the residue left in the vessel after evaporation of liquid) were determined according to *Standard Methods* (Baird and Eaton 2012). pH values were measured using a Hanna pH meter HI-207. Ammonium (NH_4^+) was determined by Direct Nessler Method (Baird and Eaton 2012), while free ammonia (NH_3) was calculated according to Oliveira et al. (2015). VFA quantification was performed by high-performance liquid chromatography (HPLC) using a Jasco HPLC (Tokyo, Japan), equipped with Rezex ROA Organic Acid H^+ column at 60 °C, UV–VIS detector and mobile phase of H_2SO_4 (2.5 mM), with a flow rate of 0.6 mL min^{-1}. Methane content was analysed on a GC-2014 Shimadzu ATF model equipped with a Porapak Q column (80-100 mesh) (2 m × 3.75 mm) with an FID detector and a flow rate of 30 mL min^{-1} of N_2 as carrier gas. The temperatures of the detector, injector and oven were of 35 °C, 110 °C and 220 °C respectively. The injected volume was of 20 µL. Sugars (sucrose, glucose and fructose) quantification was carried out using HPLC (Aminex HPX-87H IEX column, 300 mm × 7.8 mm; IR detector) operated with the following conditions: mobile phase (5 mM H_2SO_4) flow rate at 0.05 mL min^{-1}, 35 °C column temperature and 30 min retention time per sample. The injected volume was of 20 µL. The concentrations of sucrose, glucose and fructose were determined based on calibration curves obtained using the pure compounds ranging from 0.01–30 g L^{-1}. Total protein in CSL was determined by the Kjedhal method (Bradstreet 1954). Sample digestion was done on a Digester Foss, model

Table 1 Operational parameters of UASB assay

Operational parameters	Period I	Period II
Hydraulic retention time (days)	1.67	3.33
Influent COD (g L^{-1})	21.5 ± 0.5	21.5 ± 0.5
VS loading rate (VSLR) (g L^{-1} day^{-1})	8.9 ± 0.2	4.10 ± 0.02
Organic loading rate (OLR) (g L^{-1} day^{-1})	12.9 ± 0.3	± 0.02
Time (days)	0–20	20–28

Tecator/Labtec of eight tubes, whereas the distillation was performed on a distiller from Foss, Model Kjeltec 8400 Analyzer Unit. All figures were done with Prism 7 for Mac OS X (1994-2016 GraphPad Software, Inc).

Results

Characterization of the effluents

The characterization of the effluents (WaF of all formulations and WaW of Mol-CSL) resulting from the BNC fermentation with the different culture media (HS, Mol-HS and Mol-CSL) is presented in Table 2. Each parameter was measured in triplicate and the values represent the mean and standard deviation. BNC yield was quantified in all culture media.

As compared to the control (HS) the use of alternative culture media significantly improved the yield of BNC, by more than two-fold and three-fold with Mol-HS and Mol-CSL, respectively. However, as observed in Table 2, the wastewaters generated (WaF) are more concentrated in TS, VS and COD. Since Mol-CSL allowed the highest BNC yield to be obtained, it was selected for further studies related to the effluent treatment. Although the corresponding effluent (WaW), which gathers the several washing streams, is diluted as compared to WaF, all values are still well above legal limits referenced on the Portuguese legal discard limit (COD < 150 mg L^{-1}; TN < 15 mg L^{-1}; TS < 60 mg L^{-1}; sulphates < 2.0 g L^{-1}).

Biochemical methane potential of effluents generated using Mol-CSL

BMP assays (anaerobic biodegradability test) were performed to evaluate the potential of the effluents (WaF and WaW) from Mol-CSL culture medium to produce biogas. Maximum values of methane yield were achieved with both waste streams after 7 days of digestion (Fig. 2). Then, the methane yield remained constant, until methane production fulfils the criteria to finish the assay (Holliger et al. 2016).

High percentages of metanization (PM) were obtained with both waste streams (Table 3). Also, after the anaerobic biodegradability tests, the pH became neutral, within the ideal pH range for methanogenic bacteria, 6.8–7.2 (Edward et al. 2015).

Table 2 Characterization of effluents generated in the BNC production

Parameter	Units	Culture medium			
		WaF			WaW
		HS	Mol-HS	Mol-CSL	Mol-CSL
TS	g L^{-1}	26.8 ± 0.4	53.1 ± 1.3	73.4 ± 1.6	20.6 ± 0.1
VS		21.5 ± 0.4	43.7 ± 1.0	58.1 ± 1.4	13.5 ± 0.1
pH		3.36	4.35	4.35	6.80
COD		37.4 ± 5.7	54.5 ± 2.1	75.2 ± 2.5	
CODt					19.2 ± 0.2
CODs					18.8 ± 0.3
TN		1.51 ± 0.11	1.21 ± 0.01	1.73 ± 0.08	0.90 ± 10
VFAt					1.89
SO_4^{2-}	mg L^{-1}	nd	nd	883 ± 88	1830 ± 15
BNC yield	g L^{-1}	1.79 ± 0.04	4.08 ± 0.29	5.95 ± 0.76	

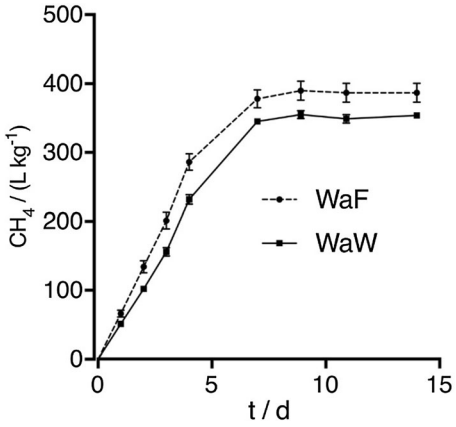

Fig. 2 Methane production (L kg^{-1} of VS) using WaF and WaW. Methane production in the blanks was subtracted from the methane produced by the assays with substrate.

VFA were not detected, suggesting that anaerobic digestion occurred without any inhibition (Appels et al. 2008). Indeed, low values of ammonia (which may cause inhibitory effects on the anaerobic digestion process when present in contents from 100 mg L^{-1} (NH_3-N) (Hansen et al. 1998) and sulphide concentrations after biodegradation were observed.

The CODs values after the biodegradability test, (0.72 ± 0.03 for WaF and 0.75 ± 0.10 for WaW) g L^{-1}, demonstrated that an overall conversion of 89.2 ± 0.1 and 88.7 ± 1.5% was achieved for WaF and WaW respectively, since each flask in the assay was initially loaded with 6.7 g L^{-1} of COD.

Table 3 Characterization of the waste streams from Mol-CSL culture medium, after anaerobic biodegradability tests

Parameter	Units	WaF	WaW
BMP	L kg^{-1}	387 ± 14	354 ± 4
PM	%	86.9 ± 3.1	79.5 ± 0.9
pH		7.17 ± 0.00	7.20 ± 0.00
CODs	g L^{-1}	0.72 ± 0.03	0.75 ± 0.10
VFA	g L^{-1}	n.d	n.d
S^{2-}	mg L^{-1}	0.023 ± 0.004	0.02 ± 0.01
NH_4^+	mg L^{-1}	312 ± 35	186 ± 10
NH_3	mg L^{-1}	9.0 ± 1.0	8.0 ± 0.0

The BMP results presented were calculated after subtraction of blank value

n.d. Non−detected

UASB reactor

Given the positive results obtained in the anaerobic biodegradability assays, a pre-feasibility study was performed for the continuous treatment of WaW, using a UASB reactor. A larger amount of WaW effluent was produced running larger fermentation batches for BNC production, using Mol-CSL medium, for 30 days. The characterization of the obtained WaW effluent was made, the following results being obtained: TS (36.7 ± 0.6) g L^{-1}; VS (14.6 ± 0.6) g L^{-1}; COD (22.2 ± 0.7) g L^{-1}; CODs (21.4 ± 0.5) g L^{-1}; pH (6.85 ± 0.2); SO_4^{2-} (1503 ± 77) mg L^{-1}. Significantly different values of TS and COD were obtained, which are assigned to the rather different conditions of the fermentation (longer time and larger scale). The methane yield, expressed as the volume of methane (L) per mass of removed COD (kg), is presented in Fig. 3a. An increasing trend was observed in Period I reaching a maximum value of about 200 L kg^{-1} on day 20. When the HRT increased (from 1.67 to 3.33 d) in Period II, the methane yield increased approaching the theoretical maximum of 350 L kg^{-1} (Fig. 3a). The maximum percentage of methane present in the biogas was approximately of 72%.

The relatively lower methane yield (expressed as the volume of methane (L) per mass removed VS (kg)) obtained in the UASB reactor in period I (102 L kg^{-1} VS) (Fig. 3b), as compared to the values obtained in the BMP (354 ± 4) L kg^{-1} VS (Table 3), is likely due to the short HRT applied. This was confirmed when the HRT was doubled in Period II and the methane yield increased to 202 L kg^{-1} VS (Fig. 3b).

Figure 4 presents the values of VS (a), COD removal (b), effluent pH (c), NH_3 (d), NH_4^+ (e), H_2S and S^{2-} (f), VFA (g) and Total Volatile Fatty acids (VFAt) (h). VS removal decreased from 90 to 62% in Period I and recovered to a higher value of 72% when the HRT increased to 3.33 d in Period II (Fig. 4-a). COD removal (Fig. 4b) increased throughout Period I and stabilized around 60%, during Period II, although the stability was not reliable, likely because of the short operation time assessed (only 7 days, corresponding to about 2 times the applied HRT). The pH was consistently above 7 and tended to increase. This led to an increase of the free ammonia Nitrogen (NH_3) during period I, as depicted in Fig. 4d. In Period I, the VFAt (Fig. 4h) achieved a total concentration of 9.5 g L^{-1}, but decreased throughout the duration of the

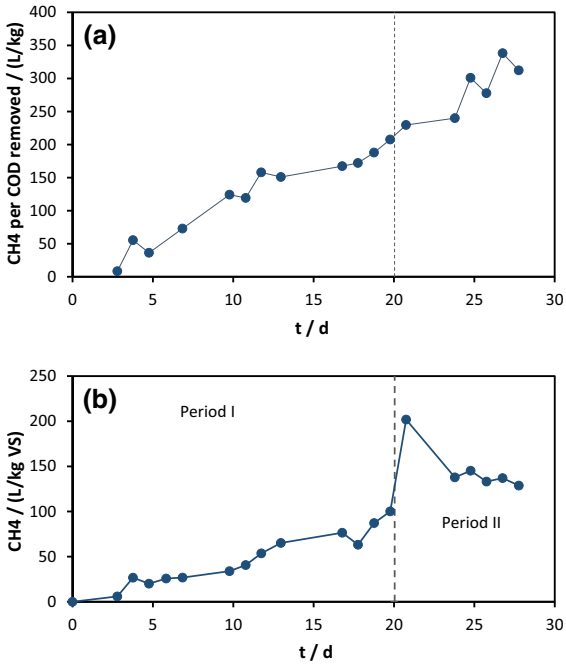

Fig. 3 Methane yield in the UASB reactor; HRT change represented after 20 days (vertical dashed line); **a** Methane yield per kg of COD removed; **b** Methane yield per kg of VS removed

assay. Acetic acid was present in the highest concentration, reaching 5.2 g L^{-1} (Fig. 4g). Therefore, this VFA accumulation at the beginning of the assay led to low methane production (Fig. 3). The concentrations of S^{2-} remained constant on period I and decreased in period II, with high oscillation. Yet, the unionised aqueous H_2S form did not increase above 100 mg L^{-1} except on days 3 (147 mg L^{-1}) and 6 (114 mg L^{-1}) (Fig. 4f).

Discussion

Molasses and CSL have high organic solids content (Battad-Bernardo et al. 2004) and contain a variety of unknown compounds, contributing to the higher compositional complexity and increased values of VS, TS and COD (Table 2). The acidic pH in all WaF effluents is explained by the production of acetic acid during fermentation with acetic acid bacteria (such as *K. xylinus*), which was mostly neutralized after washing with NaOH solutions (WaW). In addition, high concentration of sulphates (SO_4^{2-}) were observed on Mol-CSL (WaW), reaching levels well above legal limit for discharge of effluents, as also verified for COD, TS, and TN (Table 2). Therefore, all tested media need to be treated before being discarded.

Anaerobic digestion is an interesting process for effluent pre-treatment, while producing biogas, and was explored in this work (further discussed below). Soluble COD (CODs) account for > 98% of the total COD, meaning that the organic matter in the effluent is mainly soluble. Biodegradability tests of the effluents WaF and WaW were performed, where optimal conditions were used in order to maximize biogas production and COD reduction (Fig. 2 and Table 3). From these assays, high PM and low values of VFA were obtained with both effluents, confirming the high potential for biogas production (Table 3). However, VFAt was found to constitute about 10% of the COD, meaning that 90% of the COD is composed of unknown compounds. Not knowing the nature of almost all compounds, COD removal capacity is unpredictable. Also, the high concentrations of ammonia nitrogen and sulphate in these wastewaters, may inhibit the digestion process (Esposito et al. 2012). To further evaluate the BMP potential of WaW wastewaters from Mol-CSL medium, a continuous reactor UASB was used. The fast increase of methane yield as a response to the change in the HRT (from 1.67 to 3.33 d) indicates that further adjustments in the operational parameters in the UASB reactor would lead to a better performance, in terms of COD removal and methane production (Figs. 3 and 4b).

According to Souza et al. (1984), Gerardi et al. (2003) and Chen et al. (2008), sulphide values from 100 to 200 mg L^{-1} can be highly toxic, causing inhibition of anaerobic microorganisms. As observed in Fig. 4f, on both periods the sulphide (S^{2-} and H_2S form) concentrations were in the range of 100–400 mg L^{-1}. Inhibition may have occurred due to the high concentration of sulphide present throughout the UASB assay (Fig. 4f). When the ideal range of pH (7.00 ± 0.20) is surpassed (Edward et al. 2015), inhibition of the anaerobic digestion may occur due to the shift to a higher ratio of inhibitory free ammonia in the reactor (Appels et al. 2008). Subsequently, negative effects were probably observed, since concentration of free ammonia (138 mg L^{-1}) exceeded the value considered toxic to methanogenic micoroorganisms (above 100 mg L^{-1}) (Fig. 4d). As observed in Fig. 4c and d, there was an increase of pH along UASB assay, which could be related to the increase of free

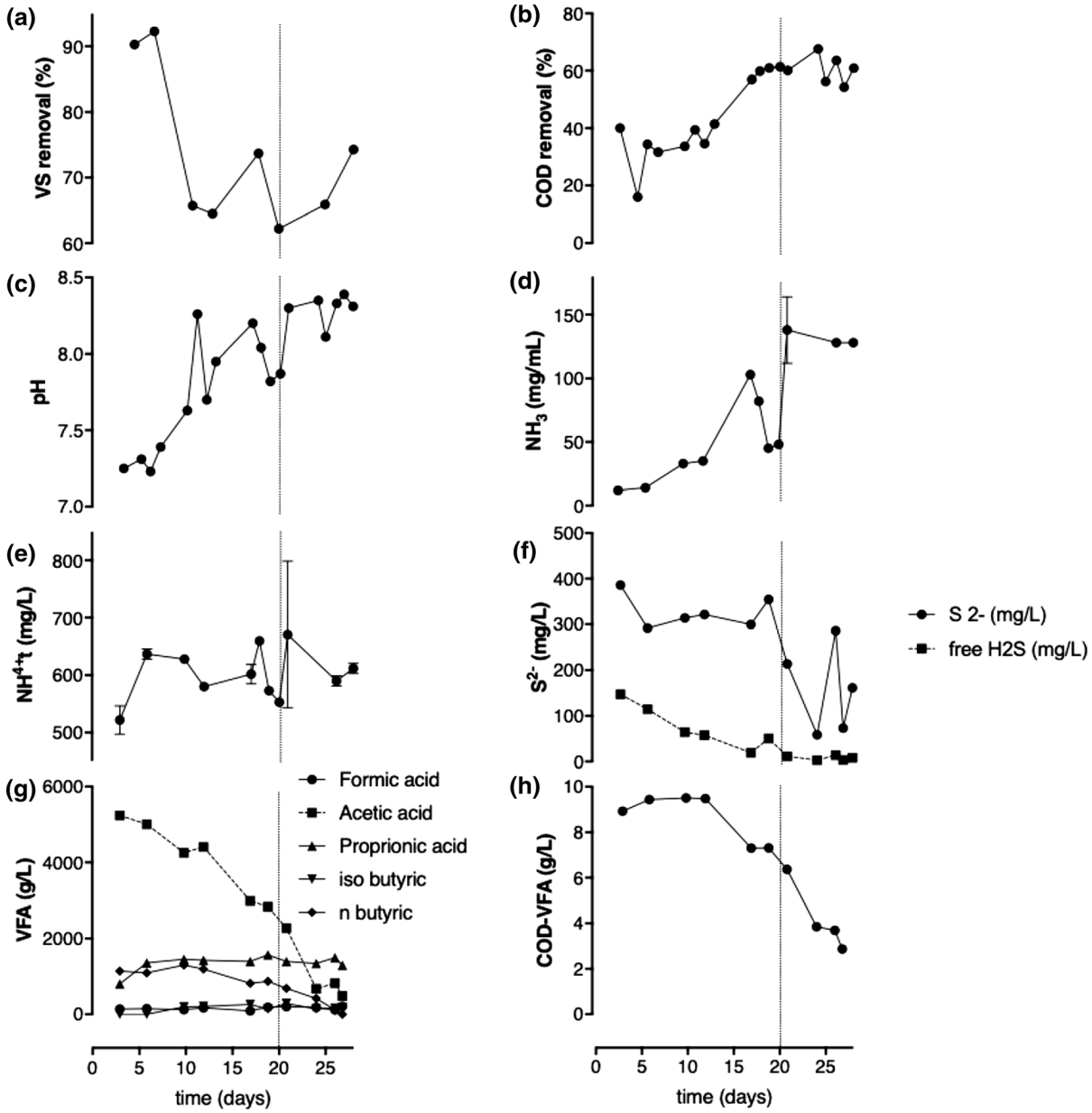

Fig. 4 UASB reactor operation; HRT change represented after 20 days (vertical dashed line); **a** VS removal (%); **b** COD removal (%); **c** pH; **d** NH_3; **e** NH_4^+; **f** S^{2-} and free H_2S; **g** formic, acetic, propionic, iso-butyric and *n*-butyric acids concentrations; **h** total VFA concentration, expressed in COD

ammonia. VFA were mainly composed of acetic acid and showed a decreasing behaviour along the two operation periods. No pH decrease was observed associated to the maximum VFA concentration detected on day 12 (9.5 g L^{-1} as COD) (Fig. 4c–h). The WaW BMP results can be compared with those obtained in the UASB reactor. In the BMP assays, ideal conditions were ensured, by adding nutrients and diluting the substrates to a COD concentration of 6.7 g L^{-1}. Contrarily, for the UASB reactor assay, the substrate (WaW) was added directly, without any dilution (initial COD value of 22.2 g L^{-1}). In fact, working with high amounts of organic matter led to an inferior performance in terms of COD removal and

biogas production. Another important factor is the HRT, as compared to the reaction time in the BMP assays. In the BMP tests, after 7 days the reaction was complete (Fig. 2), while on UASB reactor the initial HRT applied was only 1.67 d, being increased to 3.33 d, after 20 days (Figs. 3, 4).

Overall, this experiment shows the feasibility of using AD for the valorisation of the wastewaters from the BNC fermentation process. Several works, recently published, address the identification of low-cost substrates, in particular by-products from the food industry, for the production of BNC (Campano et al. 2016; Jozala et al. 2016). This is expected to increase the BNC yield while reducing the costs, and simultaneously, contribute to a sustainable circular economy. However, the studies performed so far did not address the fate of the wastewaters generated by the fermentation. For a large scale set up, this may have a rather relevant impact on the capital investment and operational costs, which must be quantified, considering the savings associated to the use of cheaper substrates and improved yields, as well as the increased costs associated to wastewater treatment. As a matter of fact, we demonstrated in this work that molasses and CSL generate an effluent with very high TS, VS and COD, as compared to the synthetic media, requiring a more demanding wastewater processing previously to being discharged. It was also shown that Mol-CSL effluents may be processed by AD, while generating biogas. In previous work, the techno-economic viability of BNC production was evaluated (Dourado et al. 2016). Given the promising results here obtained, future assays will be made to improve the performance of the UASB reactor, extending the basis for an economic analysis on the costs/benefits of a BNC production plant incorporating the production and exploitation of methane.

Funding This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2019 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and Multibiorefinery PAC (SAICTPAC/0040/2015) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020—Programa Operacional Regional do Norte. The authors thank the support through the project n° 03435: "BUILD—Bacterial cellulose Leather", funded by Fundo Europeu de Desenvolvimento Regional (FEDER) through the Programa Operacional do Regional do Norte (NORTE 2020).The authors also acknowledge the financial support of the FCT (ESF) through the grant given to J.V. Oliveira (SFRH/BD/111911/2015).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Angelidaki I, Alves M, Bolzonella D, Borzacconi L, Campos J, Guwy A, Kalyuzhnyi S, Jenicek P, Van Lier J (2009) Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci Technol 59(5):927–934

Appels L, Baeyens J, Degrève J, Dewil R (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. Prog Energy Combust Sci 34(6):755–781

Bae S, Shoda M (2004) Bacterial cellulose production by fed-batch fermentation in molasses medium. Biotechnol Prog 20(5):1366–1371

Baird R B, Eaton A D, and Clesceri L S (2012) *Standard methods for the examination of water and wastewater* (Vol. 10). E W Rice (ed) American Public Health Association, Washington, DC

Battad-Bernardo E, McCrindle SL, Couperwhite I, Neilan BA (2004) Insertion of an *E. coli* lacZ gene in *Acetobacter xylinus* for the production of cellulose in whey. FEMS Microbiol Lett 231(2):253–260

Botheju D, Bakke R (2011) Oxygen effects in anaerobic digestion-a review. Open waste Manag J 4:1–19

Bradstreet RB (1954) Kjeldahl method for organic nitrogen. Anal Chem 26(1):185–187

Campano C, Balea A, Blanco A, Negro C (2016) Enhancement of the fermentation process and properties of bacterial cellulose: a review. Cellulose 23(1):57–91

Chen L, Hong F, Yang X-x, Han S-f (2013) Biotransformation of wheat straw to bacterial cellulose and its mechanism. Bioresour Technol 135:464–468

Chen Y, Cheng JJ, Creamer KS (2008) Inhibition of anaerobic digestion process: A review. Bioresour Technol 99(10):4044–4064

Cheng Z, Yang R, Liu X, Liu X, Chen H (2017) Green synthesis of bacterial cellulose via acetic acid pre-hydrolysis liquor of agricultural corn stalk used as carbon source. Bioresour Technol 234:8–14

Costa AF, Almeida FC, Vinhas GM, Sarubbo LA (2017) Production of bacterial cellulose by *Gluconacetobacter hansenii* using corn steep liquor as nutrient sources. Front Microbiol 8:2027. https://doi.org/10.3389/fmicb.2017.02027

Costa J, Barbosa S, Alves M, Sousa D (2012a) Thermochemical pre-and biological co-treatments to improve hydrolysis and methane production from poultry litter. Bioresour Technol 111:141–147

Costa J, Gonçalves P, Nobre A, Alves M (2012b) Biomethanation potential of macroalgae *Ulva* spp. and *Gracilaria* spp. and in co-digestion with waste activated sludge. Bioresour Technol 114:320–326

Dourado F, Fontão A, Leal M, Cristina Rodrigues A, Gama M (2016) Process modeling and techno-economic evaluation of an industrial bacterial nanocellulose fermentation process. Bacterial Nanocellulose. https://doi.org/10.1016/b978-0-444-63458-0.00012-3

Dupla M, Conte T, Bouvier J, Bernet N, Steyer JP (2004) Dynamic evaluation of a fixed bed anaerobic digestion process in response to organic overloads and toxicant shock loads. Water Sci Technol 49(1):61–68

Edward M, Edwards S, Egwu U, Sallis P (2015) Bio-methane potential test (BMP) using inert gas sampling bags with macroalgae feedstock. Biomass Bioenergy 83:516–524

Esposito G, Frunzo L, Liotta F, Panico A, Pirozzi F (2012) Bio-methane potential tests to measure the biogas production from the digestion and co-digestion of complex organic substrates. Open Environ Eng J 5:1–8

Gama M, Dourado F, Bielecki S (2016) Bacterial nanocellulose: From biotechnology to bio-economy. Elsevier, Amsterdam

Gerardi MH (2003) The microbiology of anaerobic digesters. Wiley

Hansen KH, Angelidaki I, Ahring BK (1998) Anaerobic digestion of swine manure: inhibition by ammonia. Water Res 32(1):5–12

Holliger C, Alves M, Andrade D, Angelidaki I, Astals S, Baier U, Bougrier C, Buffière P, Carballa M, de Wilde V, Ebertseder F, Fernández B, Ficara E, Fotidis I, Frigon JC, de Laclos HF, Ghasimi DSM, Hack G, Hartel M, Heerenklage J, Horvath IS, Jenicek P, Koch K, Krautwald J, Lizasoain J, Liu J, Mosberger L, Nistor M, Oechsner H, Oliveira JV, Paterson M, Pauss A, Pommier S, Porqueddu I, Raposo F, Ribeiro T, Rüsch Pfund F, Strömberg S, Torrijos M, van Eekert M, van Lier J, Wedwitschka H, Wierinck I (2016) Towards a standardization of biomethane potential tests. Water Sci Technol 74(11):2515–2522

Huang C, Guo HJ, Xiong L, Wang B, Shi SL, Chen XF, Lin XQ, Wang C, Luo J, Chen XD (2016) Using wastewater after lipid fermentation as substrate for bacterial cellulose production by *Gluconacetobacter xylinus*. Carbohydr Polym 136:198–202

Jozala AF, de Lencastre-Novaes LC, Lopes AM, de Carvalho S-E, Mazzola PG, Pessoa-Jr A, Grotto D, Gerenutti M, Chaud MV (2016) Bacterial nanocellulose production and application: a 10-year overview. Appl Microbiol Biotechnol 100(5):2063–2072

Kongruang S (2007) Bacterial cellulose production by Acetobacter xylinum strains from agricultural waste products Biotechnology for fuels and chemicals. Springer, New York, pp 763–774

Lin D, Lopez-Sanchez P, Li R, Li Z (2014) Production of bacterial cellulose by *Gluconacetobacter hansenii* CGMCC 3917 using only waste beer yeast as nutrient source. Bioresour Technol 151:113–119

Massé D, Masse L, Xia Y, Gilbert Y (2010) Potential of low-temperature anaerobic digestion to address current environmental concerns on swine production. J Anim Sci 88(-suppl_13):E112–E120. https://doi.org/10.2527/jas.2009-2432

Mata-Alvarez J, Mace S, Llabres P (2000) Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresour Technol 74(1):3–16

Oliveira J, Alves M, Costa J (2015) Optimization of biogas production from *Sargassum* sp. using a design of experiments to assess the co-digestion with glycerol and waste frying oil. Bioresour Technol 175:480–485

Premjet S, Premjet D, Ohtani Y (2007) The effect of ingredients of sugar cane molasses on bacterial cellulose production by *Acetobacter xylinum* ATCC 10245. Sen'i Gakkaishi 63(8):193–199

Rajagopal R, Massé DI, Singh G (2013) A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour Technol 143:632–641

Rodrigues AC, Fontão AI, Coelho A, Leal M, da Silva FAS, Wan Y, Dourado F, Gama M (2018) Response surface statistical optimization of bacterial nanocellulose fermentation in static culture using a low-cost medium. New Biotechnol 49(2):19–27

Schramm M, Hestrin S (1954) Factors affecting production of cellulose at the air/liquid interface of a culture of *Acetobacter xylinum*. Microbiology 11(1):123–129

Souza ME (1984) Fatores que influenciam a digestão anaeróbia. Revista DAE 44(137):88–94

Tsouko E, Kourmentza C, Ladakis D, Kopsahelis N, Mandala I, Papanikolaou S, Paloukis F, Alves V, Koutinas A (2015) Bacterial cellulose production from industrial waste and by-product streams. Int J Mol Sci 16(12):14832–14849

Vazquez A, Foresti ML, Cerrutti P, Galvagno M (2013) Bacterial cellulose from simple and low cost production media by *Gluconacetobacter xylinus*. J Polym Environ 21(2):545–554

Wilkie AC (2005) Anaerobic digestion: biology and benefits. Dairy Manure Manag: Treat Handl Commun Relat NRAES 176:63–72

Zhang R, El-Mashad HM, Hartman K, Wang F, Liu G, Choate C, Gamble P (2007) Characterization of food waste as feedstock for anaerobic digestion. Bioresour Technol 98(4):929–935

Zhao H, Xia J, Wang J, Yan X, Wang C, Lei T, Xian M, Zhang H (2018) Production of bacterial cellulose using polysaccharide fermentation wastewater as inexpensive nutrient sources. Biotechnol Biotechnol Equip 32(2):350–356